Arginine to glutamine mutation in olfactomedin like 3 (*OLFML3*) is a candidate for severe goniodysgenesis and glaucoma in the Border Collie dog breed.

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Abstract

Goniodysgenesis is a developmental abnormality of the anterior chamber of the eye. It is generally considered to be congenital in dogs (*Canis lupus familiaris*), and has been associated with glaucoma and blindness. Goniodysgenesis and early-onset glaucoma initially emerged in Border Collies in Australia in the late 1990s and has subsequently been found in Europe and the USA. The objective of the present study was to determine the genetic basis of goniodysgenesis in Border Collies. Clinical diagnosis was based on results of examinations by veterinary ophthalmologists of affected and unaffected dogs from eleven different countries. Genotyping using the Illumina high density canine SNP chip and whole genome sequencing were used to identify candidate genetic regions. Expression profiles and evolutionary conservation of candidate genes were assessed using public databases. Analysis of pedigree information was consistent with an autosomal recessive mode of inheritance for severe goniodysgenesis (potentially leading to glaucoma) in this breed. There was a highly significant peak of association over chromosome 17, with a *p*-value of 2×10^{-13}. Whole genome sequences of three dogs with glaucoma, three severely affected and three unaffected dogs identified a missense variant in the olfactomedin like 3 (*OLFML3*) gene in all six affected animals. This was homozygous in all nine cases with glaucoma and 12 of 14 other severely affected animals. Of 67 reportedly unaffected animals, only one (offspring of two homozygous affected parents) was homozygous for this variant. The identification of a candidate genetic region and putative causative mutation will aid breeders to reduce the frequency of goniodysgenesis and the risk of glaucoma in the Border Collie population.

53 **Introduction**

54 Companion animals including cats, dogs and horses, suffer from a range of diseases, with
55 both genetic and environmental aetiologies. The breed barrier created by registration
56 requirements and breeding practices seeking to maximise compliance of animals with breed
57 specifications, results in increased levels of matings between relatives [1] and means that
58 purebred companion animals are particularly likely to suffer from recessive genetic
59 conditions. Understanding the genetic factors underlying these diseases is important to
60 improve welfare within breeds and the strong linkage disequilibrium in such inbred
61 populations means that small numbers of pedigree animals can be used to identify genetic
62 regions of interest, making them excellent genetic models if the same disease is present in
63 humans.

64 In response to the identification of causal variants, breeding strategies can be put in place to
65 reduce the prevalence of conditions that impact on animal welfare, especially when a
66 genetic test can be developed. However, the success of these approaches depends on
67 understanding the mode of inheritance and level of genetic contribution to the disease, and
68 ideally on identifying a causative mutation that can be used to develop a test for genetic
69 status.

70 Primary glaucoma is a condition in which increased ocular pressure damages the retinal
71 ganglion, leading to blindness (reviewed in [2]). In dogs (*Canis lupus familiaris*) it can be
72 preceded by goniodysgenesis (also known as mesodermal dysgenesis), a developmental
73 abnormality of the eye characterised by narrowing or closure of the iridocorneal angle
74 through which the aqueous humour drains. This is associated with alterations in the
75 structure of the pectinate ligament which traverses the drainage angle, called pectinate

76 ligament dysplasia (PLD) [3] (more properly pectinate ligament abnormality [4]). In the
77 Leonberger dog breed, goniodysgenesis has been associated with an increased risk of
78 developing primary closed angle glaucoma (PCAG) [2] and in Flat-Coated Retrievers,
79 development of glaucoma is positively and significantly related to the severity of
80 goniodysgenesis [5]. Goniodysgenesis is generally considered to be congenital in dogs,
81 although its subsequent progression is different among breeds. The severity is thought to
82 increase with age in Leonbergers [2] and Flat-Coated Retrievers [6] and was shown to be
83 strongly associated with age in Dandie Dinmont Terriers, Basset Hounds, Flat-Coated
84 Retrievers, Hungarian Vizslas and Golden Retrievers [7, 8]. However, goniodysgenesis
85 remained stable in Samoyeds [9] and was not associated with age in a small study of Border
86 Collies [8].

87 Sudden onset glaucoma leading to blindness with bilateral loss of eyes began to be seen in
88 young Border Collies in Australia about 15 years ago. A similar, sudden onset glaucoma in
89 young animals subsequently emerged in the UK Border Collie population, often in dogs that
90 were related to the affected Australian dogs. A number of dogs in the USA and Europe have
91 now also been diagnosed with severe goniodysgenesis and glaucoma. Goniodysgenesis in
92 Border Collies has been added to the Schedule B list of "Conditions Under Investigation" in
93 the British Veterinary Association (BVA) Eye Scheme (http://www.bva.co.uk/Canine-Health-
94 Schemes/Eye-scheme/) and it is highly prevalent in some Border collie lineages (see
95 Anadune Border collie database: http://www.anadune.com/; free access but registration
96 required). In a recent study [8], 11 of 102 Border Collies (10.8%) were reported to have
97 moderate or severe PLD (associated with goniodysgenesis). Seven (6.9%) were mildly
98 affected. Because of the association with a small number of popular sires and the high
99 prevalence in some lineages, a genetic aetiology is strongly suspected in Border Collies, but

100 the heritability and mode of inheritance of the condition is currently unknown. It is also not
101 known why a proportion of dogs diagnosed with severe goniodysgenesis go on to develop
102 glaucoma, but some do not, and nor do those with mild goniodysgenesis, over a life span of
103 15 or more years.

104 The aim of the current study was to perform a genome-wide analysis to find genetic regions
105 that are associated with severe goniodysgenesis and glaucoma in the Border Collie and
106 identify candidate mutations that might be responsible for this condition.

107

108 **Materials and Methods**

109 Full details of the Materials and Methods are available in **Supplemental File S1**.

110 *Ethical approval*

111 All studies were approved by the Veterinary Ethical Review Committee of the University of
112 Edinburgh (approval number VERC 2012-8) and the Animal Ethics Committee of the
113 University of Queensland (approval number ANFRA/MRI-UQ/565/17).

114 *Ascertainment of clinical status and pedigree*

115 Goniodysgenesis was diagnosed by examination of the eyes with gonioscopy to assess the
116 status of the drainage angle and the pectinate ligament fibres that span it. Since we had
117 results in multiple formats from many veterinary ophthalmologists, each report was
118 manually assessed to assign clinical status to each subject. Only individuals who had had
119 gonioscopy analysis were included. Breeding records and pedigree data were obtained from
120 public databases and from the breeders' websites. Websites were accessed at various times
121 up to February 2018. Full details including exclusion criteria are given in **Supplemental File**
122 **S1**.

123 *Genetic analysis*

124 DNA samples were from buccal cells collected by owners and occasionally from blood. Full
125 details of collection and extraction procedures are given in **Supplemental File S1**.
126 Genotyping used the Illumina 173k CanineHD Whole-Genome Genotyping Bead Chip
127 (Illumina, San Diego, CA, USA). Results were filtered in PLINK v1.07 [10] to remove
128 individuals that had more than 10% of missing genotypes and SNPs that had rates of
129 genotyping < 0.95, had minor allele frequency < 0.05 in this population or deviated from the

130 Hardy-Weinberg equilibrium in the controls with P value of less than 0.0001. Only the
131 autosomes were considered.

132 Cases for the analysis were defined as severely affected and consisted of dogs that had
133 goniodysgenesis of grades four or five under an early grading scheme, dogs whose
134 goniodysgenesis was described as "severe", those where the veterinary ophthalmologist
135 report indicated PLD over more than 70% of the iridocorneal angle and those that
136 developed glaucoma following a diagnosis of goniodysgenesis. Controls were dogs that were
137 assessed as clear or clinically unaffected based on at least one gonioscopy examination.
138 Previous work indicated that goniodysgenesis was not progressive in Border Collies [8] so
139 the controls were categorised on the basis of a clear gonioscopy, regardless of age. The SNP
140 analysis was performed in GEMMA v0.94.1 [11] using a linear mixed model approach,
141 controlling for relatedness using a SNP-based relatedness matrix as a random effect. The
142 significance threshold was based on a Bonferroni correction of 0.05. Additionally, a more
143 conservative, regional approach was undertaken using sliding windows of 40 adjacent SNPs,
144 overlapping by 20 SNPs in REACTA v0.9.7 [12] with the first two genomic principal
145 components controlling for relatedness. Whole genome sequencing (30X coverage) of DNA
146 from three dogs with glaucoma, three severely affected and three unaffected animals was
147 performed using the Illumina HiSeq X platform. Library insert sizes were 450 bp and paired
148 reads of 150 bp were sequenced. Reads were processed by standard procedures, detailed in
149 **Supplemental File S1** and mapped to the CanFam3.1 reference genome. The significance of
150 variants was assessed using the Ensembl Variant Effect Predictor [13].

151 *Transcriptomic analysis of candidate region genes*

152 A time course of mouse eye development from embryonic day 12 to adult was examined
153 using the FANTOM5 database [14-16] (http://fantom.gsc.riken.jp/zenbu). FANTOM5 data
154 were also used to obtain expression levels for human eye-related samples (**Supplemental**
155 **Table S1**). Transcriptomic data for the developing chicken eye were generated for another
156 project by RNA sequencing methodology (Rainger et al, in preparation). Details are given in
157 **Supplemental File S1.** Microarray-based expression data for a range of tissues in human and
158 mouse were from BioGPS (http://biogps.org) as were RNA sequencing data for chicken
159 tissues. Microarray expression data for immune cell types was derived from the
160 Immunological Genome Project (https://www.immgen.org/).

161 *Analysis of canine OLFML3 gene*

162 The region of the canine *OLFML3* gene containing the candidate causative mutation was
163 amplified by the polymerase chain reaction and the amplicon sequenced by chain
164 termination sequencing. Full details of primers and protocols are given in **Supplementary**
165 **File S1**. Sequences for analysis of nucleotide and amino acid conservation across species
166 were downloaded from Ensembl (http://ensembl.org) and alignment was performed using
167 Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/).

168

169 **Results**

170 *Familial clustering of goniodysgenesis and glaucoma*

171 Inspection of pedigrees revealed that there were three common ancestors in both the sire
172 and dam lineages for all cases of glaucoma on the goniodysgenesis database (https://bc-
173 glaucomadatabase.synthasite.com/) (N=12) including two pairs of full siblings both affected
174 with glaucoma, as well as additional cases not on the database (N=4). These common
175 ancestors dated back to the late 1970s and early 1980s and were found in multiple ancestral
176 lineages for the glaucoma cases. All the glaucoma cases had been diagnosed with
177 goniodysgenesis and subsequently had one or both eyes removed. However, several
178 individuals diagnosed with severe goniodysgenesis had not gone on to develop glaucoma.
179 This included two popular sires who reached the age of 15 years without a diagnosis of
180 glaucoma. It also included a full sibling of two dogs with glaucoma. Three dogs with severe
181 goniodysgenesis had produced a total of six offspring with glaucoma.

182 *Genome wide association analysis*

183 To identify genetic regions associated with goniodysgenesis in this breed, we performed
184 SNP analysis on 17 severely affected cases (8 female, 9 male) and 42 unaffected dogs (26
185 female, 16 male) who had been passed as "clear" at veterinary ophthalmology testing. The
186 cases all had goniodysgenesis described as "severe" (N=4), Grade 4 or 5 by the earlier
187 grading scheme (N=1 and 5 respectively), or goniodysgenesis followed by glaucoma (N=7).
188 A strong association of goniodysgenesis with a region of chromosome 17 was found (**Figure**
189 **1A**). The lowest *p*-value was for SNP rs22561716 at 17:51,919,221 ($P=2 \times 10^{-13}$) included in a
190 window of 40 SNPs that had a *p*-value of 3×10^{-07} according to REACTA. SNPs with the lowest
191 *p*-values were in a region of about 1×10^{6} base pairs (**Figure 1B**). Sixteen of the severely

192 affected and glaucoma cases were homozygous for this haplotype and one case was
193 heterozygous in this region. Within the genomic region identified by the association analysis
194 there were a number of coding genes (**Figure 1A)**, although none was known to be involved
195 in eye development or glaucoma in animals or humans. Several had strong expression in the
196 developing mouse eyeball (**Supplemental Figure F1**).

197 *Whole genome sequencing*

198 We then obtained the whole genome sequence of this region in three glaucoma, three
199 severely affected and three unaffected individuals, who were members of four nuclear
200 families (**Supplemental Figure F2**). All were distantly related through multiple lineages in
201 the extended pedigree. Relatives were chosen to highlight variants that were different
202 between affected and unaffected family members [17]. Within the region of the lowest *p*-
203 value SNP, a missense variant was found in the olfactomedin like 3 (*OLFML3*) gene, at
204 position 17:51,786,924. The mutation was c.590G>A with the predicted change of arginine
205 at position 197 to glutamine (p.R197Q). This arginine is in the olfactomedin (OLF) domain
206 [18, 19] (**Figure 2A**) and was found to be highly conserved throughout mammals, birds and
207 reptiles (**Figure 2B**). The impact predicted by the Ensembl Variant Effect Predictor [13] was
208 given as "moderate" indicating "a non-disruptive variant that might change protein
209 effectiveness", consistent with the non-lethal phenotype of homozygous animals. The SIFT
210 score was 0.55, indicating a mild effect. In humans, the mutation (c.587G>A) corresponds
211 with rs377336789 (https://www.ncbi.nlm.nih.gov/snp/) and was seen in four out of 121,412
212 chromosomes with a minor allele frequency of 3.3×10^{-5}. Genotypes are not provided for
213 the human data, but these were presumably all heterozygous, given the minor allele
214 frequency. SIFT score was 0.54 and PolyPhen score was 0.392, also consistent with a mild

215 effect. The mutation was heterozygous in four of 35 Border Collies from the Dog Biomedical
216 Variant Database Consortium (giving an allele frequency of 0.06 in this breed) but absent in
217 504 genotypes of other breeds. Notably, it was not present in 51 Leonbergers, three Basset
218 Hounds, eight Golden Retrievers or one Dandie Dinmont Terrier, all breeds reported to be
219 associated with goniodysgenesis. In addition, 350 dogs recorded as Border Collies but of
220 unknown clinical status were genotyped by Animal Genetics (Talahassie, Florida, USA) via
221 allele-specific PCR run on an ABI 3730 machine. There were 2 homozygotes and 29
222 heterozygotes among these 350 dogs, giving an allele frequency of 0.05, consistent with the
223 smaller sample from the Consortium.

224 *OLFML3 genotypes of severely affected and unaffected Border Collies*

225 The results for the mutation c.590G>A in glaucoma, severe and unaffected dogs are shown
226 in Table 1. The individual who was heterozygous for the region based on SNP chip was also
227 AG at *OLFML3*. This animal had been diagnosed with severe goniodysgenesis but did not
228 develop glaucoma over a 15-year lifespan. In addition, one unaffected dog was AA
229 genotype. This animal was the offspring of a severely affected (grade 5) father and a mother
230 diagnosed as affected (with no grade). Both parents were also AA. Under some testing
231 schemes animals could pass with some degree of goniodysgenesis [2, 4] which may be the
232 case with this dog. In general, the genotype for *OLFML3* segregated with that for
233 rs22561716, indicating strong linkage disequilibrium between them. However, an
234 unaffected mother and son were homozygous for G at *OLFML3* but heterozygous at
235 rs22561716 suggesting that the ancestral haplotype was G at *OLFML3* with T at rs22561716.
236 In addition, two unaffected individuals were homozygous for several SNPs either side of

237 *OLFML3* but heterozygous for the *OLFML3* mutation and rs22561716 and appeared to carry
238 a different haplotype.

239 To validate these results, we genotyped 31 additional Border Collies that had had
240 gonioscopy investigations: two with glaucoma (both female), four severely affected (all
241 female) and 25 unaffected (10 male, 15 female). The genotypes of these dogs are shown in
242 Table 1 and are consistent with the results suggesting that severe
243 goniodysgenesis/glaucoma was associated with homozygosity for the A allele of *OLFML3*.

244 *OLFML3 genotypes in mildly affected and uncategorised affected Border Collies*

245 Our initial analysis compared severely or glaucoma affected dogs with dogs passed as
246 unaffected at gonioscopy examination. Because of changes in the reporting scheme in the
247 UK and differences among countries, there were a number of equivocal diagnoses. These
248 were dogs where either the level of goniodysgenesis was given as mild (Grade 1 under the
249 former scheme or stated as mild or marginal on the report; N = 9; five female, four male),
250 moderate (Grade 2 – 3 under the old scheme, or stated as moderate or between 25 and
251 70% blocked on the report; N = 9; eight female, one male), or the dogs were diagnosed as
252 affected with no description given (N = 15; nine female, six male). To examine whether
253 *OLFML3* was involved in these cases we genotyped these 33 dogs. The results are shown in
254 Table 2. All three genotypes were found among these cases.

255 *Expression of OLFML3 in the eye*

256 In expression data generated by the FANTOM5 project, the *Olfml3* gene was strongly
257 expressed in mouse eyeball at embryonic and neonatal stages and declined in the adult
258 sample (**Figure 3A**). FANTOM5 data showed that the gene was expressed in human lens,

259 corneal epithelium and retina (**Figure 3B**), although the highest expression was in amniotic
260 and placental epithelial cells and in mesenchymal stem cells (not shown). *OLFML3* was also
261 expressed in the developing chick eye, with highest expression at embryonic day 6 (**Figure**
262 **3C**). In mouse microarray data (mouse probeset 1448475_at; http://biogps.org) expression
263 of *Olfml3* was highest in osteoblasts undergoing calcification, but it was also found in
264 eyecup, iris, ciliary bodies and lens (**Supplemental Figure S3A**). There was negligible
265 expression in immune system cells, except for macrophages, osteoclasts and particularly
266 microglia, which had expression comparable to lens and eyecup. Strong expression in
267 microglia was also seen in the Immunological Genome data set (**Supplemental Figure S3B**).
268 Microarray data also available through BioGPS showed strongest expression of *OLFML3* in
269 human adipocytes, uterus and retina, the only eye tissue represented (human probeset
270 218162_at; http://biogps.org) (**Supplemental Figure S3C**). RNA sequencing data compiled
271 on BioGPS revealed expression in many samples from the chicken, notably the whole
272 embryo and connective tissues and the cornea of the adult (**Supplemental Figure 3D**). Other
273 OLF family members were also expressed in the developing mouse eyeball with a similar
274 pattern to *Olfml3*, peaking in the early neonatal period and declining in the adult eye
275 (**Supplemental Figure S4**). In contrast, the myocilin gene (encoding a related protein which
276 is mutated in human open angle glaucoma) had negligible expression in embryo and early
277 neonate but increased expression at neonatal day 16 and in adult eyeball (**Supplemental**
278 **Figure S4**).

279

280 **Discussion**

281 In a number of dog breeds development of glaucoma has been associated with the presence
282 of goniodysgenesis. In the Flat-Coated Retriever, the likelihood of glaucoma was positively
283 and significantly related to the severity of goniodysgenesis [5] and in the Leonberger five
284 glaucoma-affected individuals all had a high grade of goniodysgenesis in the contralateral
285 eye [2]. An abnormal drainage angle is considered typical of some breeds, with reports of up
286 to 81% of individuals showing evidence of goniodysgenesis [9, 20]. However, the highest
287 prevalence of glaucoma reported in any single breed is only 5.52% [21], indicating that
288 severe goniodysgenesis does not necessarily proceed to glaucoma.

289 An increasing incidence of glaucoma in young animals has been reported in the Border
290 Collie breed over the last 15 years. Consistent with results in other breeds, our survey of
291 goniodysgenesis in the Border Collie found some individuals who lived entire lifetimes with
292 apparently severe goniodysgenesis without developing glaucoma. This would suggest that
293 although severe goniodysgenesis is important in the progression to glaucoma, it is not the
294 sole cause. The aetiology may involve a genetic predisposition to goniodysgenesis combined
295 with genetic, environmental or random factors influencing development of glaucoma.

296 The diagnosis of goniodysgenesis was made by veterinary ophthalmologists (such as
297 members of the BVA Eye Panel in the UK or the equivalent certifying body in other
298 countries) who performed gonioscopies to assess the status of the drainage angle and the
299 pectinate ligament fibres that cross it. Since the diagnoses were made by a number of
300 different clinicians over a period of years, there may be considerable noise in the
301 phenotype. An initial eye scheme developed by the BVA in association with the Kennel Club
302 of the United Kingdom involved assessing whether the dog had no eye abnormality (a pass),

303 or some abnormality graded from one to five according to the extent of PLD and the width
304 of the iridocorneal angle, in increasing levels of severity. Since there was a high degree of
305 inter-observer variability in assigning a grade, the scheme was subsequently amended such
306 that the dog would either pass or fail. Dogs that passed could have a drainage angle that
307 was abnormal to some extent ("mild" goniodysgenesis; see information available at
308 https://www.bva.co.uk/Canine-Health-Schemes/Eye-scheme/). The pass/fail scheme was
309 expected to have a higher level of inter-observer agreement. This was confirmed in a recent
310 study of Leonberger dogs [2] where there was a high correlation in proportion affected with
311 goniodysgenesis between a prospective study where examinations were performed by a
312 single specialist (2012-2014; 18% affected) and a retrospective study of BVA eye scheme
313 certificates (2009-2014; 22% affected). In a study of PLD in Welsh Springer Spaniels, there
314 was a good correlation between two examiners in determining unaffected eyes, although
315 there was considerable variability between examiners in assigning the degree of PLD [4].
316 This variability in categorising the extent of goniodysgenesis means that the classification of
317 phenotype as "affected" or "unaffected" can result in mild PLD or angle narrowing being
318 recorded as clinically unaffected [2, 4]. For this reason, we focussed our genome-wide
319 analysis on severely affected dogs including several that had gone on to develop glaucoma.

320 Using severely affected cases and unaffected controls, we found a strong peak of
321 association on chromosome 17, in the region of the *OLFML3* gene, where we detected a
322 missense mutation p.R197Q. Although this gene has not been implicated in goniodysgenesis
323 or glaucoma in humans, it is strongly expressed in tissues of the anterior segment of the
324 human and baboon eye including lens, iris, sclera and trabecular meshwork [18, 22] and in
325 the developing mouse eye [23] . The *Olfml3* gene was expressed throughout mouse eyeball
326 development (**Figure 3A**) and during chick eye development (**Figure 3C**). In mouse,

327 inactivation of the *Olfml3* gene by insertion of a *Lac* cassette resulted in viable and fertile
328 homozygotes [23], consistent with the relatively mild selective disadvantage of dogs with
329 goniodysgenesis. Expression has also been observed in human tissues of mesenchymal
330 origin such as bone and adipose. Olfactomedin like 3 is an extracellular matrix protein that
331 has been implicated in the epithelial to mesenchyme transition in cancer [24], in keeping
332 with its mesenchymal expression and suggesting that abnormality of this protein could be
333 associated with the formation or retention of abnormal sheets of mesenchyme in the
334 drainage angle. Olfactomedin like 3 is also proangiogenic [25] and interacts with a member
335 of the bone morphogenic protein (BMP) family, BMP4. Since BMPs including BMP4 are
336 involved in eye development (see [26] for a review), this may indicate the pathway for
337 olfactomedin like 3 action.

338 In addition, expression of *OLFML3* is also characteristic of microglia in humans [27, 28] and
339 mice (http://biogps.org; http://www.immgen.org), although it is not strongly expressed by
340 other macrophage lineages. Genetic manipulation resulting in absence of microglia is
341 associated with loss of *Olfml3* expression in mice (R Rojo et al, in revision). The retina
342 contains microglia which are important for retinal health [29, 30], and they could be one
343 source of *OLFML3* mRNA in the canine eye. Opening of the iridocorneal angle involves
344 considerable remodelling of the mesenchymal tissue within the angle, including thinning
345 and extending of the pectinate ligament and opening the trabecular meshwork [31].
346 Microglia may contribute to this process as eye development progresses by phagocytosing
347 apoptotic cells and debris from the remodelling. Abnormality of olfactomedin like 3 in the
348 microglia of the eye could interfere with this function and therefore disrupt the opening of
349 the iridocorneal angle and formation of the proper drainage channels.

350 Olfactomedin like 3 is a member of a large family characterised by the olfactomedin (OLF)
351 domain [19, 32], with several members expressed in eye structures. In particular, the
352 glucocorticoid-inducible family member myocilin (*MYOC* gene) has been implicated in
353 dominant open angle glaucoma in humans [33] and is strongly expressed in the trabecular
354 meshwork of the drainage angle [34, 35]. As with *Olfml3* mutants, there is no gross
355 phenotype in *Myoc* knockout mice [36]. Increased expression of *Myoc* mRNA resulted in a
356 reduction in *Olfml3* mRNA [37] in a mouse model, suggesting that there is an inverse
357 association between these two OLF proteins, consistent with the expression profiles in the
358 developing mouse eye (**Supplemental Figure S4**) where *Olfml3* declined in the adult while
359 *Myoc* rose. *In vitro* experiments indicated that myocilin interacts with olfactomedin 3 (also
360 known as optimedin, encoded by the *OLFM3* gene) which was co-expressed with myocilin in
361 the eye [38]. Mutation in another OLF family member, *OLFM2,* has been associated with
362 human open angle glaucoma in a small number of Japanese patients (rs779032127;
363 p.R144Q) [39] and identified as contributing to eye development [40]. These other OLF
364 family members may compensate for olfactomedin like 3 abnormalities, or may interact
365 with olfactomedin like 3 in the development of the eye. Hence genetic variation in these
366 family members may modify the impact of the *OLFML3* mutation in the Border Collies,
367 explaining the variable phenotype of homozygotes and heterozygotes carrying the
368 mutation.

369 We propose that the p.R197Q mutation in Border Collies is responsible for the presence of
370 severe goniodysgenesis predisposing to glaucoma in homozygous animals. We have also
371 seen some heterozygous animals with mild to relatively severe phenotypes (although none
372 with glaucoma), but most heterozygotes have been passed as clinically unaffected at
373 gonioscopy testing. This suggests that there may be variants at modifier loci that can

374 compensate for the *OLFML3* mutation. In particular, the presence of many OLF proteins in
375 the eye and the mild phenotype associated with relatively broadly expressed gene family
376 members suggests redundancy for at least some functions. It is also possible that
377 inadequate compensation for the putative effects of the p.R197Q mutation may be
378 responsible for the progression from severe goniodysgenesis to glaucoma.

379 There were too few glaucoma cases in our study to allow identification of loci involved in
380 progression from goniodysgenesis to glaucoma in the Border Collie and ambiguous
381 phenotyping complicates the investigation of mild goniodysgenesis. The genetic relationship
382 between the mild forms and the more severe forms is unknown, but the *OLFML3* variant
383 described here does not appear to be associated with most cases of mild goniodysgenesis
384 indicating that genetic predisposition to mild goniodysgenesis may be independent of the
385 findings of this study. Although developing a test for the *OLFML3* mutation would allow
386 breeders to select against homozygotes to decrease the prevalence of the risk genotype for
387 severe goniodysgenesis, it is important not to reduce the breeding pool too much because
388 of the risk of other recessive conditions resulting from homozygosity for alleles that are
389 identical by descent. Therefore, we need to understand why some of our cases progressed
390 to glaucoma at a young age and some did not before we can make recommendations to the
391 breeders of Border Collies worldwide.

392

393 **Author contributions**

394 KMS, CAP and LLF conceived the study. AJC, LLF and KMS curated and prepared the DNA
395 samples. OM, SCB and LLF performed initial genetic analysis. LLF and CAP performed the
396 SNP analysis. CAP and SJB performed the genome wide sequencing analysis. KMS and AJC

397 designed, performed and analysed the *OLFML3* genotyping. JJS analysed the data from the
398 Dog Genome Consortium. AdeK and CW analysed the Animal Genetics samples. KMS
399 analysed the public gene expression data. JR and VT-R created and analysed the chicken
400 eye data. KMS, LLF and CAP drafted the text and all authors read and edited the final
401 manuscript.

402

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415

416 **Conflict of Interest**

417 AdeK and CW are employed by Animal Genetics, Talahassie, FL, USA, which is now offering a
418 test for genetic predisposition to severe goniodysgenesis based on these results.

419

420 **Figure legends**

421 **Figure 1.** Genome wide association with severe goniodysgenesis and glaucoma

422 **A.** Manhattan plot for the canine genome; chromosome 17 is shown in more detail and
423 genes in the region are shown below.

424 **B.** Homozygosity within the region of interest. Each row is a DNA sample and each column
425 shows a SNP. Red and yellow indicate homozygosity for the major and minor allele
426 respectively and green shows heterozygosity. The upper block shows severely affected and
427 glaucoma animals; the lower block shows those passed as clinically unaffected at
428 gonioscopy testing. The most significant SNP is shown as yellow in the cases (homozygous
429 for the minor allele); one clinically unaffected animal was homozygous for this allele and
430 one severely affected animal was heterozygous.

431

432 **Figure 2.** Conservation of *OLFML3* predicted amino acid sequence

433 **A.** Amino acid sequence of the canine OLFML3 protein. Functional domains are highlighted:
434 blue – signal peptide; pink – coiled coil domain; yellow – olfactomedin like domain [18, 19].
435 Within the yellow region, bold lettering shows the core OLF region. Green shows the
436 arginine at position 197.

437 **B.** Conservation of arginine 197 (green) in OLFML3 of vertebrates. Arginine is found at the
438 equivalent position in all mammal, bird and reptile species for which there is an annotated
439 *OLFML3* gene, and in coelacanth but not in other fish. The region around this arginine is also
440 highly conserved.

441

442 **Figure 3.** Expression of *OLFML3* genes in eye regions

443 **A.** Expression of *Olfml3* in developing mouse eyeball. Results are based on CAGE and were
444 downloaded from the FANTOM5 database (http://fantom.gsc.riken.jp/5/tet). Y axix shows
445 RLE normalised values. Light grey – embryonic samples; dark grey – neonatal samples; black
446 – adult sample.

447 **B.** Expression of *OLFML3* in human eye tissues and cells. Results are based on CAGE and
448 were downloaded from the FANTOM5 database. Y axis shows RLE normalised values. Light
449 grey – optic nerve and retina; mid grey – cornea; dark grey – lens.

450 **C.** Expression of *OLFML3* in the developing chick eye. Results are based on RNA sequencing.
451 Y axis shows transcripts per million (TPM) values. Light grey – embryonic day 5; mid grey –
452 embryonic day 6; dark grey – embryonic day 7.

453

454 **Supplemental Material**

455 **Supplemental Table S1.** FANTOM5 samples used in analysis of gene expression. Details can
456 be found at fantom.gsc.riken.jp/5/sstar/.

457 **Supplemental Figure S1**. Expression of genes in the candidate region in the developing
458 mouse eye. Upper panel shows the canine genes in the region. Lower panel shows
459 expression of the genes in the developing mouse eyeball. Y axis shows RLE normalised
460 expression levels. Data taken from FANTOM5 (see **Supplemental Table S1**). Light grey –
461 embryonic; dark grey – neonatal; black – adult.

462 **Supplemental Figure S2.** Pedigrees of animals used for whole genome sequencing. Grey fill
463 indicates severe goniodysgenesis; black fill indicates glaucoma; white fill indicates
464 unaffected or unknown status. Double line indicates a first cousin mating; all glaucoma
465 individuals can be traced back to the same three distant common ancestors on both sides.
466 Animals with a BC ID were sequenced.

467 **Supplemental Figure S3.** Expression of olfactomedin like 3 gene in cells and tissues

468 A. Expression in mouse tissues and cell lines (http://biogps.org; microarray probeset
469 1448475_at). Y axis shows relative intensity of expression (arbitrary fluorescence
470 units).
471 B. Expression in mouse myeloid cells (http://www.immgen.org, dataset "MFs,
472 Monocytes, Neutrophils"; microarray probeset 10500808). Y axis shows relative
473 intensity of expression (arbitrary fluorescence units).
474 C. Expression in human tissues and cell lines (http://biogps.org; microarray probeset
475 218162_at). Y axis shows relative intensity of expression (arbitrary fluorescence
476 units).
477 D. Expression in chicken tissues (RNA sequencing data curated from public databases
478 and analysed using Kallisto, available at
479 http://biogps.org/dataset/BDS_00031/chicken-atlas/; Bush et al, in revision). Y axis
480 shows tags per million (TPM).

481 In A, C and D, blue – nervous tissues; dark green connective tissues; light green
482 digestive tract and renal; yellow – reproductive; orange – immune; red – ocular.

483 **Supplemental Figure S4.** Expression of olfactomedin family genes in the developing mouse
484 eye (data from FANTOM5; http://fantom.gsc.riken.jp/zenbu/). Y axis shows RLE normalised

485 expression levels based on CAGE analysis. Light grey – embryonic; dark grey – neonatal;

486 black – adult.

487

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597

598

599

Genotype Clinical status	AA	AG	GG	TOTAL
SNP-chip dogs[1]				
Glaucoma	7	0	0	7
Severe goniodysgenesis	9	1	0	10
Unaffected	1	19	22	42
Other dogs				
Glaucoma	2	0	0	2
Severe goniodysgenesis	3	1	0	4
Unaffected	0	17	8	25
TOTALS				
Glaucoma	9	0	0	9
Severe goniodysgenesis	12	2	0	14
Unaffected	1	36	30	67

600

601 **Table 1.** Genotype frequencies for *OLFML3* mutation c.590G>A in severely affected and unaffected

602 dogs.

603 Dogs that were included in the genome wide analysis (SNP-chip dogs) are shown separately from

604 dogs that were only tested for *OLFML3*.

605

Genotype Clinical status	AA	AG	GG	TOTAL
Mild	3	3	3	9
Moderate	4	2	3	9
Affected, no grade	7	3	5	15
TOTALS	14	8	11	33

606

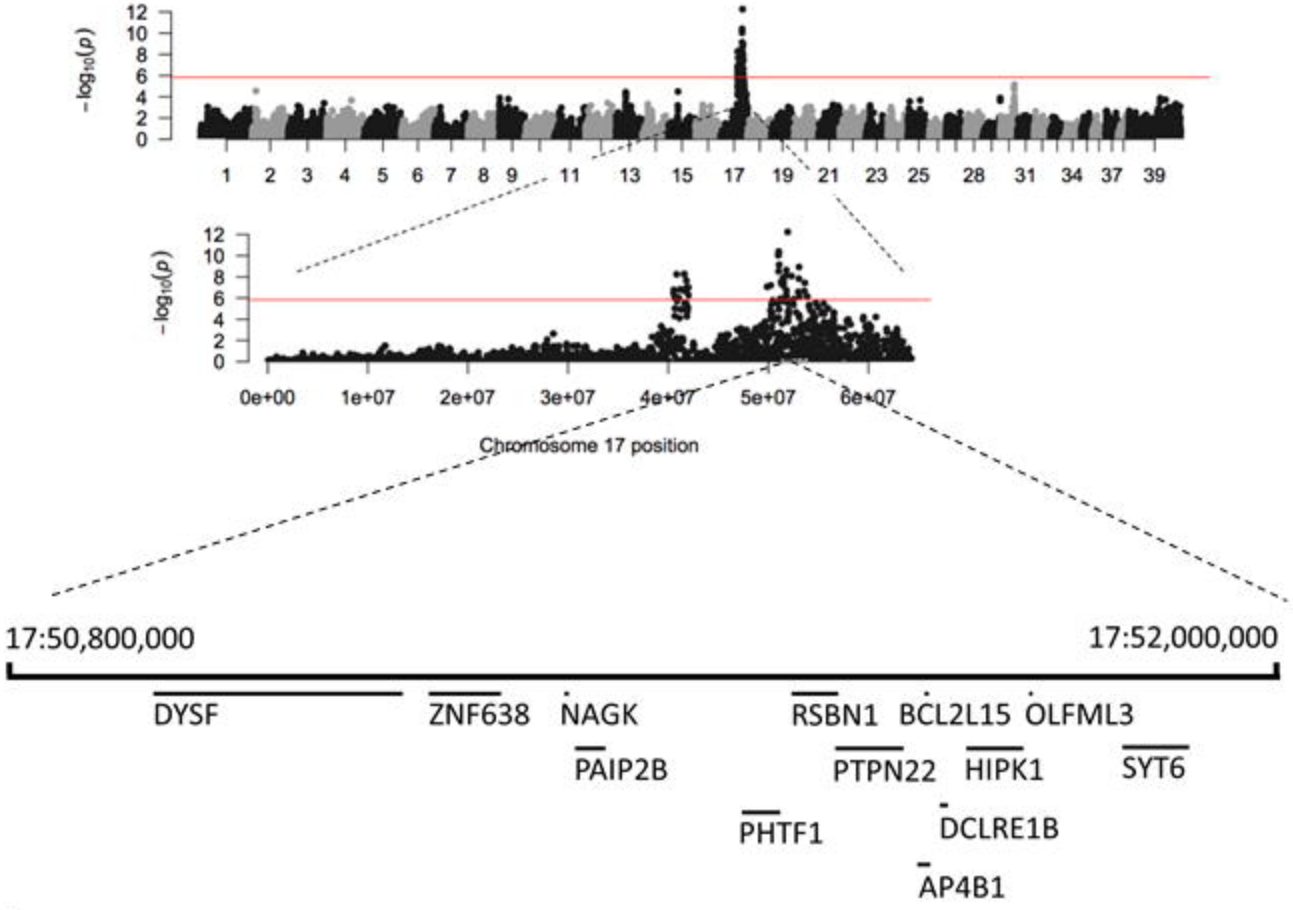
607

608 **Table 2.** Genotype frequencies for *OLFML3* mutation c.590G>A in mild, moderate and

609 uncharacterised affected dogs.

610

A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10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 11 13 15 17 19 21 23 25 28 31 34 37 39
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Chromosome 17 position
17:50,800,000
17:52,000,000
DYSF
ZNF638
NAGK
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PAIP2B
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HIPK1
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DCLRE1B
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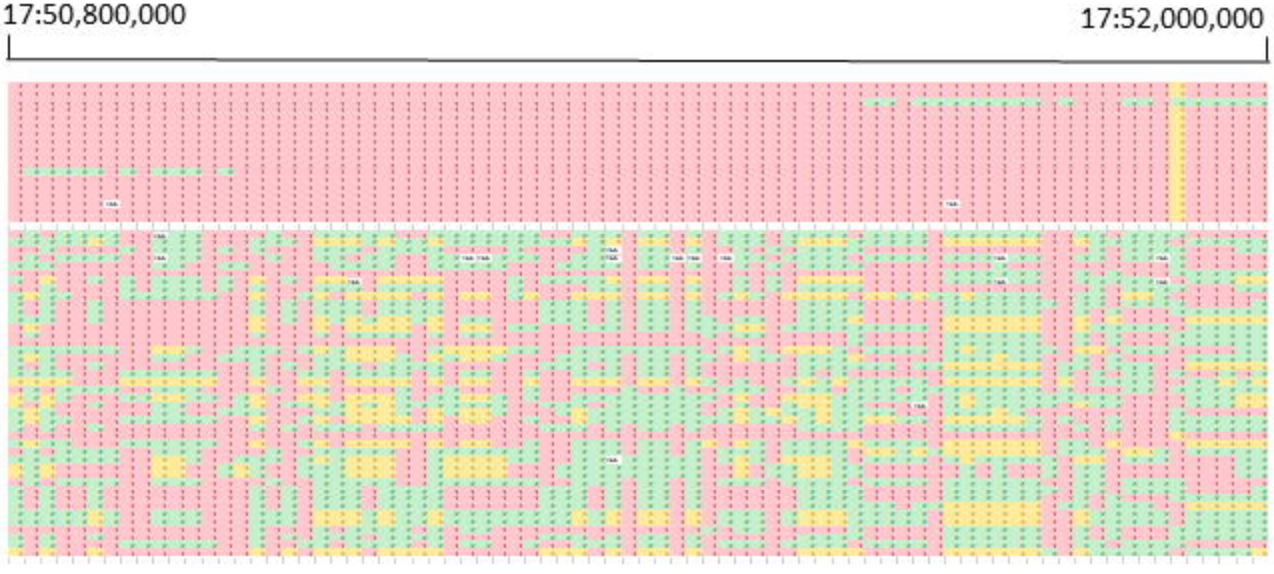
B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A

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KGKGRRNEKYDMLTDCGYTISQVRSMKILKRFGGPAGLWTKDPMGPTEKIYVLDGTQNDT

AFVFPRLRDFTLAMAARKASRVRVPFPWVGTGQLVYGGFLYYARRPPGGPGGGGGLENTL

QLIKFHLANRTVVDSSVFPAEGLIPPYGLTADTYIDLAADEEGLWAVYATREDDRHLCLA

KLDPQTLDTEQQWDTPCPRENAEAAFVICGTLYVVYNTRPASRARIQCSFDASGTLTPER

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B

```
Opossum_OLFML3          VRSMKILKRFGSPAGMWTKDPLGPTEKIFVLDGTQNDTAYVFPRLRDFTLSTTARKASRV
Wallaby_OLFML3          VRSMKILKRFGSSAGMWTKDPLGPSEKIFVFDGTQNDTAYVFPRLRDFTLSSAARKASRV
Tasmanian_Devil_OLFML3  VRSMKILKRFGSSAGMWTKDPLGHSEKIFVLDGTQNDTAYVFPRLRDFTLSTAARKASRV
Shrew_OLFML3            VRSMKILKRFGGPTGLWTKDPLGPTEKIFVLDGTHNDTAFIFPRLRDFTLAMAARKAARV
Kangaroo_rat_OLFML3     VRSMKILKRFGGPAGMWTKDPLGPAEKIYVFDGTQNDTALVFPRLRDFTLAMAARKASRI
Hedgehog_OLFML3         VRSMKILKRFGSPPGLWTKDPLGTTEKIYVLD-PQNDTAFIFPRLRDFTLAMAARKASRV
Armadillo_OLFML3        VRSMKILKRFGGPAGLWTKDPLGPAERIYVLAGTQNDTALVFPKLRDFTLATAARKASRV
Mouse_Olfml33           VRSMKILKRFGGSVGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRI
Rat_Olfml3              VRSMKILKRFGGSAGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFTLTMAARKASRI
Elephant_OLFML3         VRSMKILKRFGGPAGLWTKDPLGPTEKIYVLDGTQNDTAFVFPRLRDFSLSTATRKASRV
Guinea_pig_OLFML3       VRSMKILKRFGGPAGLWTKDPLGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Hyrax_OLFML3            VRSMKILKRFGGPAGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFSLSMATRKASRV
Microbat_OLFML3         VRSMKILKRFGGSAGLWTKDPLGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Squirrel_OLFML3         VRSMKILKRFGGPAGLWTKDPLGPTEKIYVLDGTQNDTAFVFPRLRDFTLTMAARKASRV
Pig_OLFML3              VRSMKILKRFGGPAGLWTKDPLGPTEKIFVLDGTQNDTAFVFPRLRDFTLAMAARKASRI
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Dog_OLFML3              VRSMKILKRFGGPAGLWTKDPMGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Ferret_OLFML3           VRSMKILKRFGGPAGLWTKDPMGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Panda_OLFML3            VRSMKILKRFGGPAGLWTKDPMGPTEKIYVLDGTQNDTAFIFPRLRDFTLAMAARKASRV
Cat_OLFML3              VRSMKILKRFGGPAGLWTKDPMGPAERIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Mouse_lemur_OLFML3      VRSMKILKRFGGPAGLWTKDPLGPTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Human_OLFML3            VRSMKILKRFGGPAGLWTKDPLGQTEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Cow_OLFML3              VRSMKILKRFGGPAGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Sheep_OLFML3            VRSMKILKRFGGPAGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFTLTMAARKASRV
Dolphin_OLFML3          VRSMKILKRFGGPAGLWTKDPLGPAEKIYVLDGTQNDTAFVFPRLRDFTLAMAARKASRV
Coelacanth_OLFML3       IKSMKILKRIGSSTGMWARDPLGDSDNIYVLEGTGNDTVYEYSRARDFTQSFVMR-ARRI
Xenopus_OLFML3          VTAMKILKRFGSSAGLWTKDLAGNSDRIYVFDGAGNDTVYVYPRMKEFTLSSSTRKAAKI
Chicken_OLFML3          VRAMKILKRFGSSAGLWTKDAAGSSEKIYVFDGTANDTVYIFPRMREFTLFSATRRAARI
Flycatcher_OLFML3       VRAMKILKRFGSASGLWTKDAAGSSEKIYVFDGTANDTVYVFPRMREFTLFSATRKAARI
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Turtle_OLFML3           VKAMKILKKFGSSAGLWTKDPVGSSEKIYVFDGTSNDTVYVFPRMREFTLFSATRKAARI
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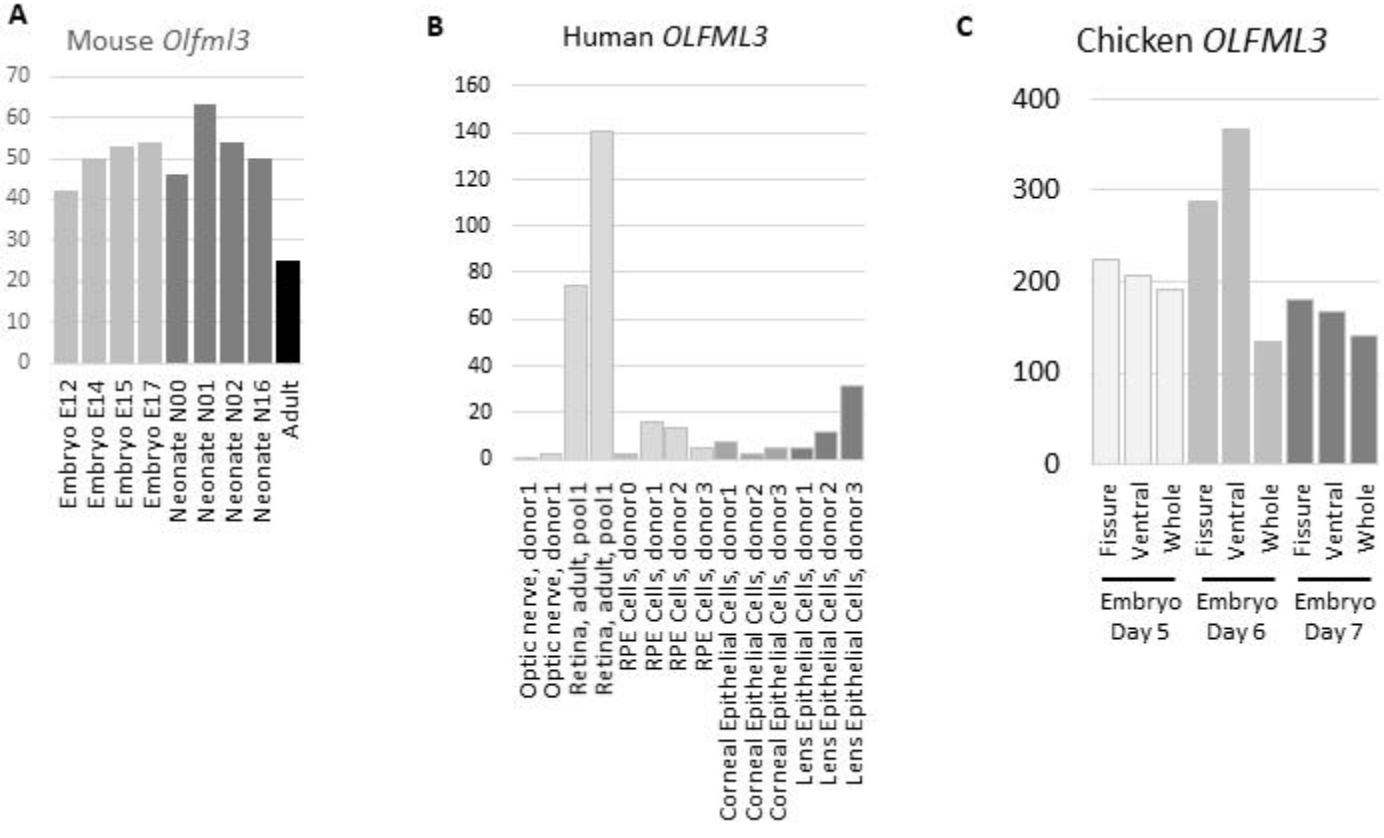
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Mouse Olfml3
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30
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Neonate N00
Neonate N01
Neonate N02
Neonate N16
Adult
B
Human OLFML3
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140
120
100
80
60
40
20
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RPE Cells, donor3
Corneal Epithelial Cells, donor1
Corneal Epithelial Cells, donor2
Corneal Epithelial Cells, donor3
Lens Epithelial Cells, donor1
Lens Epithelial Cells, donor2
Lens Epithelial Cells, donor3
C
Chicken OLFML3
400
300
200
100
0
Fissure
Ventral
Whole
Fissure
Ventral
Whole
Fissure
Ventral
Whole
Embryo Day 5
Embryo Day 6
Embryo Day 7